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MULTIDISCIPLINARY STUDY OF RADIONUCLIDES AND HEAVY METAL CONCENTRATIONS IN WILDLIFE ON PHOSPHATE MINED AND RECLAIMED LANDS

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FLORIDA INSTITUTE OF PHOSPHATE RESEARCH

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FINAL REPORT

1985 PILOT STUDY

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PERSPECTIVE

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Few studies have been conducted of radionuclides in wildlife populations inhabiting areas disturbed by phosphate mining in Florida. Those that have been undertaken have generally been limited in scope by the relatively high cost of radiological analyses. As a result, almost no data are available for most organisms. Audubon's plan in carrying out this project, then, was to complement the work of other investigators studying the radiation environment associated with phosphate mining by studying groups of animals that had theretofore not been examined. Audubon selected for study two aquatic reptiles (alligators and snapping turtles) and one terrestrial mammal (armadillos) based on the criterion that these species have significant proportions of their mass comprised of bony tissue that would likely show elevated concentrations of radium if, in fact, uptake were a problem. Animals from mined, phosphate mineralized, and unmineralized land in central Florida were targeted for sampling.

Soon after the study got underway, however , it became clear that snapping turtles were not abundant in mine-related terrains. Instead, Audubon researchers collected softshell turtles of the genus Trionyx, which attain high densities in mined areas. The plan also called for sampling up to thirty individuals of other species that might be of interest. Because of the rather high radium concentrations shown by hardshell turtles of the genus Pseudemys (Florida cooter) analyzed early in the study, Audubon emphasized further analysis of cooters in their opportunistic sampling.

The results of Audubon's analyses varied considerably between species. The alligator bones contained only low concentrations of radium, and there were no significant differences between alligators collected from mined, mineralized or unmineralized land. Like the alligators, armadillos showed no significant differences in radium concentrations between sites.

The hardshell turtles did show differences depending upon where they were obtained. Those sampled from mining-impacted land had, on average, seven times as much radium as those sampled from unmineralized habitat. Like the hardshell turtles, the softshell turtles showed differences in radium activity depending upon where they were captured. Lowest average concentrations were observed in individuals collected from unmineralized land. Turtles from mineralized, unmined land had the next highest concentrations, followed by the specimens from mined land. Even though the average levels in the bony tissue of softshell turtles collected from mined land were the highest of all landforms, they were still extremely low.

The radiochemical literature includes sporadic data on ashed bone samples for many species in many locations. Radium concentrations range from 0.05 to 12 pCi/g; the average levels reported from Florida's phosphate area are in the low to moderate portions of the reported range. Intensive radionuclide analyses of wildlife generally are only performed when there is reason to believe that animals have been exposed to high levels or radioactivity.

EXECUTIVE SUMMARY

The purpose of this project was to evaluate the degree, if any, of radionuclide enhancement in the tissues of certain Florida vertebrate species on lands impacted by phosphate mining. "Impacted" was interpreted to include both clay settling ponds and also natural lakes and ponds adjacent to and, at least historically, subject to runoff from mined areas. The species selected included the alligator (*Alligator mississippiensis*), the Florida softshell turtle (*Trionyx ferox*), and the nine-banded armadillo (*Dasypus novemcinctus*), and provision was also made for inclusion of opportunistically-collected specimens of other vertebrate species. Control collecting sites included both mineralized (phosphate-bearing) but unmined and non-mineralized areas.

The specimens ultimately collected included 26 alligators, 26 softshell turtles, and 30 armadillos, with the addition of one river otter (*Lutra canadensis*), five red-bellied cooter turtles (*Pseudemys nelsoni*), eight peninsula cooter turtles (*Pseudemys floridana peninsularis*), and one Florida snapping turtle (*Chelydra serpentina osceola*), opportunistically collected.

Mine-impacted collection sites were all in Polk County. Important sites included Lake Hancock and settling pond N12A on property of the IMC Company; various minor sites are listed in Appendix A. Mineralized unmined sites included Lake Manatee (Manatee County) and Lake Buffum (Polk County), as well as minor sites also listed in Appendix A. Specimens from sites considered unmineralized were drawn principally from Lake Griffin (Lake County) and Lake Okeechobee (mainly Palm Beach County). The terrestrial species (armadillos) were collected where encountered, with no single site of intensive collecting.

For each specimen collected, a standardized, carefully cleaned, bony or largely bony sample was removed and subjected to ashing in an electric kiln with progressively increasing temperature ultimately reaching 900°C. The resulting ashed specimens were pulverized and subjected to analysis of gross alpha and beta activity, gamma spectroscopy, radium assay (by the radon-emanation method), and, in some cases, to Inductively-Coupled Plasma (ICP) analysis for heavy metals. When the latter proved insufficiently sensitive, we substituted analysis by furnace and ion probe for selected target elements (barium, arsenic, fluorine, and lead), chosen for reasons of known toxicity, probable occurrence in settling ponds, and uniformity with those selected by other investigators. We found only low levels of arsenic and barium, and these were therefore omitted after a small number of analyses.

The results of the radium assays were the most significant. The mean level of ^{226}Ra in bony tissues of alligators from mine-impacted sites was found to be 0.87 pCi/g (N = 10), compared with 0.87 pCi/g (N = 6) for mineralized-unmined sites, and 0.80 pCi/g (N = 10) for unmineralized sites. In one specimen, a tooth-bearing section of the jaw was analyzed as well as the normal vertebral sample, and was found to have approximately four times the ^{226}Ra content of the "standard" sample. Despite this intriguing single result, however, the alligators showed no statistically significant enhancement of bone ^{226}Ra on mine-impacted lands.

The data for armadillos were somewhat difficult to interpret, because of the great range in gross alpha found even within a single site-category. The mean gross alpha activity found for specimens from mined lands was 3.22 (N = 10), with overall range of 0.8 to 10.0 pCi/g - For mineralized-unmined lands the corresponding figures were 3.41 (N = 10), range 0.0 to 10.1 pCi/g), and for unmineralized lands 1.09 pCi/g (N = 10), range 0.0 to 1.9. Only seven radium assays were conducted on armadillo samples in view of the erratic variation found in the gross alpha counts. These Ra results were analyzed statistically by the NPAR1WAY (non-parametric) technique, and found not to be statistically significant.

For softshell turtles, the results: were somewhat complex. Mine-impacted specimens showed ^{226}Ra concentration of 1.82 pCi/g (N = 10, range 0.8 to 2.8). Specimens from mineralized-unmined lands had average concentration of 1.05 pCi/g (N = 6), range 0.0 to 1.6, and those from unmineralized land averaged 1.22 (N = 10), range 0.4 to 3.3. Certain control specimens showed elevated ^{226}Ra concentrations, probably as a result of fertilizer pollution in suburban areas. Nevertheless, enhancement of ^{226}Ra in the mine-impacted animals was statistically significant, even though our series from this category was living in an essentially macrophyte-free environment and were feeding upon insects. Elsewhere this species will feed heavily upon aquatic vegetation, as we found for the Lake Griffin specimens. In view of the results for hard-shelled turtles (below) and the unusually high ^{226}Ra concentrations sometimes shown by aquatic plants in phosphate areas (Upchurch, et al., 1981), we would thus anticipate the possibility of significant Ra enhancement in softshells from mine-impacted sites.

The highest radium elevations were found in the hard-shelled turtles (*Pseudemys nelsoni* and *Pseudemys floridana peninsularis*). These opportunistically-collected specimens were collected only from mine-impacted and from unmineralized lands. Data for the two species were pooled in view of our small sample sizes and the very similar morphology and ecology of the two species.

The *Pseudemys* in unmineralized sites yielded a mean ^{226}Ra concentration in the ashed bone of 1.10 pCi/g (N = 5), range 0-9 to 1.2. The mining-or they may be carried as low-concentration accessories in mildly radioactive but more abundant minerals such as zircon, sphene, or apatite. Larsen and Phair (1954) compiled data demonstrating the average concentrations of alpha-generating radionuclides (uranium, thorium, and radium) in several broad categories of igneous rocks.

The deposition of uranium in carbonate-type sedimentary rocks is variable, but in many cases deposition in such rocks is sparse to non-existent (Bell, 1954). Organisms of several plant and animal phyla extract $CaCO_3$ from marine solution. Some, such as algae, do not require dissolved oxygen whereas molluscs and corals do; generally high concentrations of dissolved oxygen are unfavorable to the precipitation of uranium, although corals are known to fix dissolved uranium in an oxidized form (uranyl iron). However, in the formation of phosphate as opposed to carbonate-type deposits, uranium tends to be deposited simultaneously and in considerably higher concentrations.

In Florida, the phosphate concentrations are quite localized; the distribution of the reserves is shown by Fernald (1981). Several non-contiguous areas occur along the western half of the northern and central parts of the Florida peninsula, but by far the most important deposits, from the commercial viewpoint, are in the so-called Bone Valley Formation, occupying a large area of Polk, Hardee, Hillsborough, and Manatee Counties, and a small area of northern DeSoto County. Major, although deeper and thus less accessible, reserves also exist in eastern Florida, for example in the St John Platform deposits, and indeed some modern stratigraphers estimate that only a small proportion of Florida phosphate occurs in the Bone Valley Formation.

The uranium in the Bone Valley deposits is normally prevented from impacting surface systems by an overburden of sand, about 3-15 meters in thickness, capped by a lesser but variable thickness of topsoil. Thus, while the concentration of uranium, measured as U_3O_8 equivalent, is of the order of 100 to 200 ppm in the phosphate deposits themselves (Altschuler et al., 1956; Cathcart, 1956), the topsoil may be as low as 5 ppm, and the sand overburden 20-30 ppm. These zones, however, are subject to leaching, especially of the radium which is the daughter isotope most likely to be found in water-soluble forms, and below the sand overburden the leachate may concentrate over a relatively narrow range of depths (up to 3 m) to levels of 100 to 300 ppm U_3O_8 equivalent.

The action of phosphate mining brings these radionuclides-into the surface strata in much higher concentrations than would normally be found in the topsoil, from which they can be drawn into the tissues of surface biota. Moreover, while most elements of the uranium series are metallic elements that are likely to remain in the matrix in which they are generated (with the exception of some migration of radium, and perhaps polonium and lead, through aqueous transport), the radionuclide produced directly by radium breakdown, namely Radon-222, is a chemically inert gas that may diffuse out of the matrix and into adjacent systems in the course of its relatively short half-life of 3.82 days. Atoms of radon taken into the lungs of terrestrial vertebrates in the course of respiration will in most cases be exhaled in the course of the breathing cycle. However, the high atomic weight of this isotope, and the short half-life, result in an appreciable proportion of the atoms residing in the lungs long enough to break down (via alpha emission). This produces a series of subsequent decay products, many of them highly radioactive and breaking down further to subsequent daughter isotopes within minutes, whilst two of these radon-daughters, ^{210}Pb and ^{210}Po, with half-lives of 20 years and 138 days respectively, are likely to remain in significant amounts in the organism for the rest of its life, even if no further radon is taken into the lungs.

Radium-226 is the isotope most commonly measured in the course of attempts to assay hazards to living organisms resulting from natural (or artificially enhanced) radioactivity. The selection of radium has several justifications:

1. It has a half-life of 1620 years, which is sufficiently long for its occurrence to be considered permanent rather than transitory when incorporated into living tissues or strata separate from the point of generation, but short enough for it to be a highly radioactive element so that its concentration may be evaluated even when it is present in very small amounts.

2. Its concentration may be measured conveniently and accurately by means of the radon emanation technique.

3. Of the various uranium-decay isotopes, radium is the one most susceptible to concentration in living tissues because it is a Group-II (alkaline earth) element, susceptible to absorption along with dietary calcium in vertebrates, and laid down in bony tissues that have a very slow rate of biological turnover as compared to soft tissues. Similar reasoning justifies the special attention given to Strontium-go, a product of nuclear fission, in evaluating biological effects of radioactive fallout.

After ^{226}Ra, ^{210}Po may be the most critical radioisotope to evaluate in organisms. Polonium-210 is not normally present in concentrations correlated with its ^{210}Pb precursor because of the very different chemistries of the two elements. Moreover, in radiation dosimetry of the ^{210}Pb, ^{210}Bi, ^{210}Po series, the alpha particle emanating from the ^{210}Po during its decay over a half-life of 138 days contributes more than 95% of the biologically effective radiation dose (Holtzman, 1969).

Various studies have evaluated radium concentrations of both "normal" and "enhanced" soils, waters, and organisms. The "enhancement" resulted from a variety of causes, ranging from natural radionuclide concentrations caused by proximity to thorium or uranium ores, to artificial Concentrations resulting from phosphate-mining activities, fallout, or improperly sealed radioactive waste disposal. Holtzman (1969) studied concentrations of ^{226}Ra, ^{210}Po, and ^{210}Pb in aquatic fauna in ecosystems not considered to show abnormal concentrations of these isotopes. The organisms studied included marine and freshwater fishes, molluscs, seals, and whales. Radium-226 concentrations were fairly stable at around 5 pCi/100 g bone ash and 0.2

pCi/100 g soft tissue. However, concentrations of ^{210}Pb and ^{210}Po were generally higher than those of ^{226}Ra. One interesting comparison was obtained by comparing the ^{210}Pb concentrations of the teeth of sperm whales with the bone of baleen whales. The former were found to have about ten times the ^{210}Pb concentrations of the latter. Nevertheless, the usefulness of this result is reduced by the uncontrolled variables (i.e., both the tissue and the species were different); but it is noteworthy that in the course of our study (see below), a tooth-bearing sample from an alligator snout had several times the ^{226}Ra concentration of a bony (neck) sample from the same animal.

deBertoli and Gaglione (1972) evaluated the concentration of ^{226}Ra in soils from areas thought to be non-enhanced in radionuclide concentration. They found an overall range of 0.08 to 3.8 pCi per gram. They also noted that the freshwater fish they sampled had radium concentrations ranging from 1.4 to 3.2 pCi/kg, and concluded that the fish demonstrated a "bioconcentration" factor of 10 to 22 in relation to the radium in the ambient water, although radium concentrations of the food organisms of the fish were not evaluated.

Rope and Whicker (1985) studied the concentration of ^{226}Ra in edible fish from waters adjacent to uranium surface mining activity. They reported concentrations of 12-33 pCi/liter in the water of these ponds, in which Ca was present at 30-330 mg/l. The flesh of trout introduced to the ponds showed activity of 6.3 to 30 pCi/kg wet weight. The greatest concentration of radium was found in the bone, but high concentrations were also found in the skin and fins, and only 6% in the flesh; the bone showed a concentration of up to 260 times that of the flesh. The distribution of radium was found to be rather similar to that of ^{89}Sr in Tilapia. In this introduced population, Rope and Whicker found that the concentration of radium in the trout increased with exposure time; by contrast, Parsont (1967) and Swanson (1983) found a decrease, or no change, in radium concentration with age in the fish they studied.

Kitchings et al. (1969) discussed the possibility of bioconcentration of radionuclides passed up through food chains. They found that bioconcentration depended heavily upon the element under consideration. For example, deer showed a negative bioconcentration ratio of ^{134}Cs and ^{137}Cs (i.e. 0.4) relative to their forage items, whereas bobcats feeding upon rabbits and cottonrats showed respective bioconcentrations of radioactive caesium of 6.1 to 13.9 and 6.9 to 18.7 respectively. Rabbits feeding upon grass concentrated ^{90}Sr by a factor of 8.4 in their bone, while caribou concentrated ^{90}Sr by a factor of 7 in their bone, but reduced it (to 0.02) in their muscle. To explain differences in concentrations he found, Kitchings et al. emphasized the importance of taking into account the biological as well as the physical half-life of radionuclides ingested by organisms, and observed that nuclides such as ^{90}Sr are lost very slowly because of their tendency to concentrate in bone, while tritium was cycled out of the organisms rather quickly, although it was, as one might have expected, retained for longer periods in desert animals.

Potential hazards to humans living on reclaimed phosphate lands in Florida have been discussed amply in the popular press (e.g. St. Petersburg Times, 2/8/85; Tampa Tribune 2/8/85), and stem from various accounts in the technical literature. Ryan et al. (1983) summarized information on the effects of radioactivity on individuals living in houses on reclaimed phosphate lands, and Randolph (1983) summarized information on cancer and genetic risks associated with ionizing radiation. Walsh and Lowder (1983) described health risks associated with radon in indoor situations. Watson (1983) discussed food chain transport of ^{210}Pb and ^{210}Po from soil to parts of crops eaten by humans, and from animal feed to meat. In a paper much discussed in the popular press, Lyman (1985) established a correlation between leukemia incidence and radium concentration in well water in 27 Florida counties, finding that the ten counties with the highest concentrations of radium in well water had 1.5 to 3 times the incidence of leukemia found in the seventeen counties with the lowest radium concentrations.

In addition to the possible effects of radionuclide concentrations upon humans in Florida in phosphate mining areas, a limited amount of investigative effort has attempted to document the concentration in other vertebrate species, in most cases species consumed by man. Studies by O.C.Boody of Environmental Science and Engineering of Gainesville, Florida, involve evaluation of radiological parameters of water, sediments, and biological tissue (fish, zooplankton, macroinvertebrates, and aquatic macrophytes) in twelve reclaimed and four natural lakes. Exchange of radioelements between phosphatic strata and the hydrologic environment (involving ^{238}U, ^{234}U, ^{220}Th and ^{226}Ra) in both unmined and mined-over areas are being studied by J.K.Osmond and J.B.Cowart of Florida State University. Another current study by Post, Buckley, Schuh, and Jernigan is evaluating radioactivity in food grown on Florida phosphate lands. Three of the most significant and relevant of the recent studies are those of Upchurch et al. (1981), of O'Meara et al. (1983), and of Montalbano et al. (1983). The first of these studied ^{226}Ra concentrations in freshwater fish in Central Florida. In these organisms, all species studied showed some degree of bioconcentration of radium relative to the ambient water, although not necessarily with respect to the substrate, and no evidence of biomagnification was found, i.e., there was no tendency for carnivorous fish to show higher radium concentrations than the herbivorous species. These authors established that, in general, fish of small species showed higher radium concentrations than larger ones, possibly because they had a higher proportion of bone in the body, but within a single species (specifically the bluegill), the larger individuals had higher radium concentrations, presumably because of progressive increase of relative skeletal development with ontogeny. However, perhaps the most dramatic discovery revealed by this study was the high concentration of radium in aquatic vegetation. Despite careful cleaning of the samples, concentrations of 25,937 pCi per kilogram and 92 954 pCi per kilogram were identified for Elodea and filamentous algae, respectively, and the fish that fed directly upon these plants showed the highest concentrations of radium in their tissues.

O'Meara et al. (1983) studied radium concentrations in several species of aquatic birds, the species selected (Wood and Mottled Ducks, Moorhens, and Double-crested Cormorants) including both herbivorous and piscivorous species. In these species, radium was consistently higher in bony than in soft tissues. Bone ash derived from the duck species averaged 4,440 pCi/kg in the Central Florida clay settling ponds, while the piscivorous cormorants averaged only 441±44 pCi/kg. Moorhens averaged 445±29 pCi/kg. The substrate of the settling basins showed an activity of 23,400±3,200 pci/kg, while off-site control substrates showed only 200±100 pCi/kg. Thus, despite the high concentration of radium in the ducks, the concentration was actually reduced relative to that of the substrate. Waterfowl skeletons contained four times (i.e. 800 pCi/kg) of the recommended tolerable maximum according to human standards (Federal Radiation Council, 1961), although O'Meara et al. considered this acceptable in view of the naturally short life span of waterfowl.

Montalbano et al. (1983) studied concentrations of ^{226}Ra and of lead, arsenic, fluorine, and barium in waterfowl on phosphate-mined lands. They found an average concentration of 3.08 pCi per kilogram in muscle tissue of ducks on such sites, as compared with an off-site value of 0.86 pCi/kg (control specimens being from lake Okeechobee). Concentrations of the other elements (Pb, As, F, Ba) were not found to be statistically different between mined and control areas. These authors established that the condition of the waterfowl, as measured by depth of muscle and fat tissues, did not show any correlation with the radionuclide concentration shown by the individual, and concluded that the existing concentrations presented no threat to the health of humans eating duck meat, in reasonable quantities, from the phosphate-mined areas.

The purpose of our own study was to build further upon the data generated by the above three studies, and to evaluate radionuclide concentrations in certain species, not previously studied, widespread in Florida, that are at least occasionally utilized for human consumption. One of the species, the American alligator, is used increasingly as a "novelty" food in Florida, the flesh being a by-product of the growing hide industry based upon this now substantially-recovered species. A second species, the Florida softshell turtle, Trionyx ferox, has long been a favorite food species in Florida, and specimens from the phosphate-mined areas are not only commonly eaten by man, but are also sold commercially in Bartow and elsewhere. However, in order to broaden our range of species, we included herbivorous turtle species of the genus Pseudemys in our study. These two species, by analogy with results obtained by Upchurch et al. with herbivorous fish, might be expected to concentrate significant amounts of radium in their extensive bony tissues. Our studies also included the armadillo, a terrestrial mammal, widespread both on and off phosphate-mined lands in Florida, with relatively high body proportions of bony tissue, and of omnivorous and opportunistic feeding habits. For the purpose of our studies, then, this last species was a sort of "terrestrial equivalent" of the aquatic but also omnivorous and opportunistic Florida softshell turtle.

STATISTICAL ANALYSES

All organisms were grouped according to the nature of the environment or the substrate from which they were collected. This gave us three groups each for *Alligator mississippiensis*, *Trionyx ferox*, and *Dasypus novemcinctus*. The *Pseudemys* samples, not having been initially included in our "target" species, were smaller and less comprehensive, and were allocated to only two substrate types (mine-impacted and unmineralized).

The grouped samples were tested for statistically valid differences by the use of the NPAR1WAY procedure included in the SAS computer software package. The analyses were carried out by the University of Central Florida Institute of Statistics. This procedure performs analysis of ranks and certain rank scores of a response variable across a one-way classification. NPAR1WAY is a nonparametric procedure for testing that the distribution of a variable has the same location parameter across different groups. Wilcoxon scores were derived and compared using a Kruskal-Wallis test and a p value was calculated.

Radium-226 concentrations in ashed tissue samples from the alligators showed no significant difference at the .05 confidence level, (p value = 0.2386), when compared using this test procedure. Similarly, no statistically valid differences in ^{226}Ra concentrations of ashed tissue samples were found among the three groups of armadillos, (p value = 1.000).

The comparison of ^{226}Ra concentrations in the softshell turtles proved to be more interesting. When the samples were compared a significant difference was found at the .05 confidence level, (p value = 0.0431). A Multiple Comparisons test was performed to locate the differences within the three *Trionyx ferox* groups. This test procedure found no difference between the ashed tissue samples from the unmined and unmineralized lands. It did, however, find both of these samples to be significantly different from the samples collected from mine-impacted lands.

Two composite samples of *Pseudemys*, (P. *nelsoni* and P. *floridana peninsularis*), were also analyzed for ^{226}Ra concentrations in their ashed tissues. The sample from the mine-impacted lands consisted of three specimens of *P. f. peninsularis* and five of *P. nelsoni*. The sample from unmineralized areas consisted of five *P. f. peninsularis* and none of P. *nelsoni*. The ^{226}Ra concentrations in these two groups showed a rather dramatic difference, reflecting an approximately seven-fold enhancement in the mine-impacted areas. This difference was highly significant at the .01 confidence level, (p value = 0.0034).